Cryptosporidium Serology in Human Populations [Project #438]

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OBJECTIVES:
The first objective of this project was to determine the sensitivity of the second-generation enzyme linked immunosorbent assays (ELISA) developed at the Centers for Disease Control and Prevention (CDC) and of a previously described immunoblot assay. After that, the researchers planned to address some issues related to specificity of the ELISA. Then, the goal was to analyze changes in levels of antibody reactivity over time in persons with laboratory-confirmed cryptosporidiosis. The next objective was to compare initial results of the ELISA and immunoblot at a community level and further assess the usefulness of the ELISA at a population level by testing sera of residents of six communities, some of which had waterborne outbreaks of cryptosporidiosis. Finally, the researchers planned to evaluate the usefulness of saliva specimens for the detection of antibodies to Cryptosporidium using the ELISA assay.

BACKGROUND:
The serological detection of antibodies to Cryptosporidium has emerged as a potential tool for population level studies. Second generation ELISA methods are known to detect immunoglobulin G (IgG) to two Cryptosporidium sporozoite surface antigens (apparent molecular mass 17 and 27 kDa). During 1996, 1,292 cases of cryptosporidiosis were reported to public health in the province of British Columbia in western Canada. This rate, seven times higher than that in 1995, was due to waterborne outbreaks occurring in this province. It was recognized that collection of serum specimens for study of the host immune response would be important and sera were collected from 41 adult volunteer cases associated with these outbreaks. A total of 232 sera were collected over a 24-month period. These Kinetics Cases sera were used to assess the host immune response. Sera from residents of six communities, some of which had outbreaks of cryptosporidiosis due to contamination of their drinking water, were also tested to determine the usefulness of serology at a population level.

HIGHLIGHTS:
A serological approach appeared to be a useful epidemiological tool to assess cryptosporidiosis at the population level. The host immune response to both Cryptosporidium antigens was documented. The second generation CDC ELISA was the optimal format. The 17 kDa assay, in which the IgG response diminishes within 12 months, appeared to be a useful tool for studying recent events while the 27 kDa appeared to detect cumulative events. When applied to six communities (outbreak and non-outbreak), the seroprevalence within the communities varied. Outbreaks were detectable in some communities.

APPROACH:
In phases 1 and 2 of the project, testing was carried out on sequential sera collected from patients infected during waterborne outbreaks of cryptosporidiosis and other unique sera collected from patients with other parasitic infections. The sensitivity and cross reactivity of both ELISA and immunoblot (miniblot) assays were assessed, although because populations not exposed to Cryptosporidium were not available, this assessment was seen as a comparative evaluation, not an assessment of quantitative accuracy. The ELISA was compared to results of the immunoblot in phase 2. In phase 3, the ELISA assay was used to test 1,944 sera already tested by immunoblot and 2,150 sera from six communities. It was expected that new information from this project would include an estimate of the usefulness of Cryptosporidium antigens as markers of recent infection, an estimate of the sensitivity, and a comparative assessment of agreement combined with some cross-reactivity data of both the CDC ELISA and the immunoblot. The new information was also expected to include an assessment at a population level of seroprevalence of antibodies to Cryptosporidium in outbreak and non-outbreak settings.

RESULTS/FINDINGS:
Inter-laboratory studies showed that the ELISA method produced consistent results in different laboratories. ELISA results for the same sample sets from two different laboratories showed excellent correlation. The ELISA procedure behaved reliably once the technology was transferred. The ELISA method detected all laboratory confirmed cases at 10 or more weeks post onset. ELISA IgG test results showed improved sensitivity over the immunoblot test results. No cross reactivity between Cryptosporidium and either Giardia or Toxoplasma was observed when sera from laboratory confirmed cases were compared. The ELISA was the format used to test community sera. Using the ELISA 17 kDa assay, seropositivity peaked at the 11 to 20 week interval post onset and decreased with time after infection to a negative detection limit at the 51 to 60 week interval post onset. The community serological responses detected the outbreaks but lagged the epidemic curve by two months. Analysis of community ELISA data showed significant differences across all communities.

IMPACT:
Serological testing could be used to characterize communities or populations in terms of exposure levels to Cryptosporidium. A serological approach may also be useful in predicting health impacts of parasite contamination in different communities.
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